PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
40263		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI2003/000705	29.09.2003	27.09.2002
International Patent Classification (IPC) or national classification and IPC		
C12N 15/10, C12N 15/62, C12N 15/64, C07K 7/06, C07K 7/08		
Applicant		
CTT Cancer Targeting Technologies OY et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22.04.2004	23.12.2004
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Form PCT/IPEA/409 (cover sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000705

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

- ☐ international search (under Rules 12.3 and 23.1(b))
- ☐ publication of the international application (under Rule 12.4)
- ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☒ the international application as originally filed/furnished

☐ the description:

pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ the drawings:

pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000705

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority was considered valid for the features disclosed in document "Combinatorial Chemistry & High Throughput Screening, volume 6, 2003, Mikael Björklund et al: 'Use of Intein-Directed Peptide Biosynthesis to Improve Serum Stability and Bioactivity of a Gelatinase Inhibitory Peptide', pages 29-35". Therefore, this document is not included in the statement in Box V.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2003/000705

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-23	YES
	Claims		NO
Inventive step (IS)	Claims	12,17-22	YES
	Claims	1-11,13-16,23	NO
Industrial applicability (IA)	Claims	1-23	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Documents cited in the International Search Report:

D1: Gene, Volume 231, 1999, Sibylle Mathys et al: "Characterization of a self-splicing mini-intein and its conversion into autocatalytic N- and C-terminal cleavage elements: facile production of protein building blocks for protein ligation", pages 1-13

D2: The Journal of Biological Chemistry, Volume 271, no. 36, 6 September 1996, Shaorong Chong et al: "Protein Splicing Involving the Saccharomyces cerevisiae VMA Intein", pages 22519-22168

D3: The Journal of Biological Chemistry, Volume 274, no. 7, 12 February 1999, Thomas C. Evans et al: "The in Vitro Ligation of Bacterially Expressed Proteins Using an Intein from Methanobacterium thermoautotrophicum", pages 3923-3926

D4: The Journal of Biological Chemistry, Volume 274, no. 26, 25 June 1999, Thomas C. Evans et al: "The Cyclization and Polymerization of Bacterially Expressed Proteins Using Modified Self-splicing Inteins", pages 18359-18363

D5: Gene, Volume 192, 1997, Shaorong Chong et al: "Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element", pages 271-281

D6: WO 9947550 A1

D7: WO 0036093 A2

D8: Biochemistry, Volume 40, 2001, Fouroozan Mohammadi et al: "Protein-Protein Interaction Using Tryptophan Analogues: Novel Spectroscopic Probes for Toxin-Elongation Factor-2 Interactions", pages 10273-10283

D9: Current Opinion in Biotechnology, Volume 11, 2000, Francine B Perler et al: "Protein splicing and its applications", pages 377-383

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

Novelty

D1-D3 disclose the use of intein-fusions in order to produce peptides. The intein-mediated cleavage is induced by shifts in temperature and pH. The cleavage is not induced by any thiol reagents and would therefore not affect any disulphide bridge that may exist in the peptide. (D1: abstract; page 5, column 2, paragraph 2; page 8, column 2, paragraph 3; D2: abstract; page 22164, column 2, paragraph 2; figure 4; D3: abstract; page 3924, column 1, paragraph 4-column 2, paragraph 2.)

The applicant argues that the cleavage in D2 is induced with thiol reagents in addition to pH/temperature changes. However, the cleavage in figure 4A seems to only be induced with pH and temperature.

Documents D4 and D5 also disclose the use of intein-fusions in order to produce peptides. The intein-mediated cleavage is induced by shifts in temperature and pH. However, the cleavage is, in addition to pH and temperature, also induced with thiol reagents and would therefore affect any disulphide bridge that may exist in the peptides due to the reducing effect on disulphide bridges that thiol reagents possess. (D4: abstract; page 18360, column 1, paragraph 3; D5: abstract; figure 2; page 277, column 1, paragraph 2.)

In D1-D3, a number of different peptides are expressed. There is nothing in these documents that indicates that the expressed peptides contain any disulphide bridge. Even if the peptide originally contains disulphide bridges, the intein-mediate expression might affect the disulphide bridges. However, it is still possible that some of the expressed peptides contain one or more disulphide bridges. In that case, the subject matter claimed in claim 1 and some of the dependent claims will lack novelty. In other cases, an inventive step must be shown (see arguments below).

Inventive step claim 1

The applicant's arguments are focused on the aspect of "small" peptides and the usefulness of the method for expressing "small" disulphide bridge containing peptides, which have different characteristics compared with larger peptides. This might be true, but the claims are not restricted to "small" peptides, except for some of the claims that are acknowledged novelty and inventive step. In addition, it can be mentioned that no definition of the word "peptide" has been found in the description.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

D1 is one document disclosing the closest prior art.

The *only* difference between the method as claimed in claim 1 and the method disclosed in D1 is that the method in claim 1 produces a peptide, regardless of its length, having at least one disulphide bridge.

This difference gives rise to a way of expressing peptides having at least one disulphide bridge.

The problem to be solved is thus to be able to express peptides, regardless of their length, having at least one disulphide bridge.

In view of D1, it is known that intein-mediated cleavage can be induced by only using changes in temperature and pH. It is well known that peptides comprising disulphide bridges are sensitive to the conditions in their surroundings. Some conditions may affect the disulphide bond and thus the activity of the protein. Different temperatures and pH-values do not affect a disulphide bridge to any great extent and it is obvious for a person skilled in the art that the method in D1 is suited for expressing peptides having disulphide bridges, since the intein-mediated cleavage is induced under such mild conditions. Consequently, the subject matter claimed in claim 1 is considered to lack an inventive step.

Inventive step claims 2-11, 13-16 and 23

The independent claims relating to combinations of intein-mediated expression with other methods, e.g. phage display and the use of auxotrophic cells, give rise to methods for screening, analyzing and improving the peptides obtained from phage display selections. This is, according to the applicant, a very tedious process today and the present method would facilitate this procedure.

This might be true. However, the combination of intein-mediated expression with e.g. phage display or incorporation of unnatural amino acids does not give rise to any unexpected effect that is not already known for these different applications (e.g. phage display and the use of auxotrophic cells).

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

It seems as the applicant, for solving the problem of tediousness, only has combined well known techniques with already well known advantages in order to create a less tedious process. This can not be acknowledging an inventive step.

Therefore, the additional aspects claimed in claims 2-11, 13-16 and 23 are considered to be detailed executions obvious for a person skilled in the art. Some of the aspects are already mentioned in D1 and other aspects such as phage display, libraries and the use of auxotrophic hosts for incorporating unnatural amino acid are well known techniques for a person skilled in the art. (See e.g. D7: abstract; page 32, line 2-page 38, line 6; claims; D8: abstract.) Thus, the subject matter claimed in claims 2-11, 13-16 and 23 is considered to lack an inventive step.

The applicant also argues that the methods claimed in claims 16 and 23 are particularly suitable for expressing peptides with certain properties, e.g. improved solubility. However, as the claims are worded, they are not restricted to such applications but relate merely to the production of peptide with unnatural amino acids in general.

D2 and D3 are additional documents considered to disclose the closest prior art. These might be used in a similar manner to D1 in order to examine the lack of inventive step of claims 1-11, 13-16 and 23.

D6 discloses the native CTT peptide. (Page 6, line 20.)

D9 is an article disclosing inteins and applications thereof.